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Notes on natural history and call description of the Critically Endangered *Plectrohyla avia* (Anura: Hylidae) from Chiapas, Mexico

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Plectrohyla avia Stuart, 1952 was described on the basis of a single male from Granja Lorena, Quetzaltenango, Guatemala (Stuart 1952). Since then, only a few observations have been made of this species, expanding the distribution from the vicinity of the El Triunfo Biosphere Reserve in the Sierra Madre del Sur de Chiapas southeast across the Volcán Tacaná of Chiapas and the Volcán Tajumulco of southwestern Guatemala to the highlands of the Quetzaltenango District of Guatemala (Duellman and Campbell 1992). Only general information on morphology and distribution has been published, and the natural history of this species remains unknown (Duellman 2001; Kohler, 2010).

Plectrohyla avia is the largest member of its genus; males reach 90.4 mm, and females 70.4 mm. None of the large species of *Plectrohyla* (*P. avia*, *P. teuchestes* Duellman and Campbell 1992*; P. exquisita* McCrannie and Wilson, 1998; *P. hartwegi* Duellman 1968) have been tested molecularly (Faivovich 2005; Frost 2006; Pyron and Wiens 2011; Duellman et al. 2016), and thus it is not possible to establish relationships with the smaller species of the genus. The recent split of *Plectrohyla*, describing *Sarcohyla* for the *bistincta* group (Duellman et al. 2016) and retaining *Plectrohyla* for the *guatemalensis* group, helps to clarify the taxonomic panorama. All four species of large *Plectrohyla* are considered Critically Endangered by the IUCN (Acevedo and Smith 2004; Cruz et al. 2010; Santos-Barrera et al. 2004, 2006). While they are likely all closely related, *P. avia* stands out within the group as it has a single prepollical spine (bifid on the others) and very protruding teeth.

No detailed natural history observations on any of the larger species of *Plectrohyla* (*P. avia*, *P. teuchestes*, *P. exquisita*, and *P. hartwegi*) have been published (Duellman and Campbell 1992; Duellman 2001; Köhler 2010). We herein report observations on the reproductive activity and natural history of *P. avia* in its habitat.

On May 5th, 2016, we arrived at the small village of Mirador Chiquihuites, Municipality of Unión Juárez, Chiapas, at ±1700h, just as it was starting to rain (amongst the first rains of the season, according to the locals). Several *Plectrohyla* cf. *sagorum* Hartweg, 1941 were calling from a small ravine below the road. The ravine was located at 2015 m asl at the following coordinates: 15.095167°, -92.106669° datum = WGS84.

About 150 m upstream, within a narrow ravine

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Fig. 1. Aquatic axillary amplexus of *Plectrohyla avia*. (Right top). *Photo by César L. Barrio-Amorós*. **Fig. 2.** Male of *Plectrohyla avia* grasping the female's head with its long and curved teeth. (Left top). *Photo by César L. Barrio-Amorós*. **Fig. 3.** Amplectant pair of *Plectrohyla avia*, two motionless males and eggs over the pool. (Bottom left). *Photo by César L. Barrio-Amorós*. **Fig. 4.** Reproductive male of *Plectrohyla avia* inside a hole on the waterfall side, from where it calls. (Bottom right). *Photo by César L. Barrio-Amorós*.

covered by dense canopy, we heard a low but distinctive call (see below). Upon closer examination, we observed five large green frogs in a small pool. The pool was approximately one square meter in area and located at the base of a three m high waterfall with a very light flow. The five large green frogs (four adult males and one adult female) were *Plectrohyla avia*. Upon discovery all five animals were apart from each other and below the surface of the water. Two males were noticeably larger than the other two, and at one point engaged in what appeared to be male-male combat. Two of us (CG and HF) saw one male grasping the other and though not very clear (too dark), we saw how the largest embraced the smaller and forced it to escape. After this we filmed a short video (https://youtu.be/aa2O-BguqOY). Shortly after the combat, the largest male embraced the only female in an axillar amplexus (Fig. 1), while the other three males remained inactive under water. This male was significantly larger than the female, as has been reported for some large *Plectrohyla* (Duellman and Campbell 1992; Köhler 2010). During the first minutes the female moved around the pool while in amplexus with the inactive male (Fig. 2). The female appeared to be trying to escape from the male who remained in strong amplexus. After some minutes swimming across the pool, beneath the surface of the water at all times, the female stopped on the edge of the pool. The amplectant male remained motionless. Another male began calling underwater (sec 16 of video; the male was not seen calling directly but was the only close male and it was under water; other underwater calls were heard afterwards occasionally); immediately thereafter the amplectant male began scratching the top of the female's head with the long teeth that protrude from the upper jaw. The female tried to release herself from the amplecting male and again began swimming, but the male continued to move his head laterally scratching the female's head. While being passed by the swimming female, the other males remained motionless (Fig. 3). The pair again stopped at the other edge of the pool. The rocky bed and walls of the pool were covered by large eggs, all underwater, probably from earlier amplectant pair(s). We did not see the female laying eggs.

Above the small pool with the five adults, we observed three more adults. Two males were calling from within small holes in the splash-zone of waterfalls varying from one to four m in height (Fig. 4). Another adult male was perched on the wet wall of the higher waterfall (Fig. 5). Adult males called from within holes, crevices or beneath the surface of the water.

Two kinds of eggs were observed in the pool. A smaller one in dense quantities with pigmented pole, and other bigger and unpigmented, more scattered and in a much lower density. We cannot rely of which one was laid by *P. avia*, as we did not see directly the female laying eggs. *Plectrohyla* cf. *sagorum* was present on the ravine as well (we saw several calling males and

Fig. 5. Active male of *Plectrohyla avia* on a waterfall wall, the only male we saw outside the water or holes. *Photo by César L. Barrio-Amorós.*

one amplectant pair) and we cannot be sure to which species the eggs belong. Only unpigmented eggs have been reported for *Plectrohyla* (Duellman and Campbell 1992). An alternative explanation could be that the larger unpigmented eggs belong to different stages of development of the same species.

Vocalization

Recording specifications: The first call (Fig. 6) was recorded with a Sony RX10 camera in HD 1080, and sound extracted in a WAV file, 48 kHz of sample rate, 16 bit signed. The second call (Fig. 7) was recorded with a Nikon d5100 camera and sound extracted in a WAV file, 44.1kHz of sample rate, 16 bit signed. Recordings were analyzed in Raven Pro 1.5 Beta (Bioacoustics Research Program 2013), with a Hann function window, FFT 1024 samples, and 50% overlap). A summary of spectral and temporal features of vocalizations of *Plectrohyla avia* is in Table 1, and details of each pulse of the calls are in Table 2.

Vocalization description: The male advertisement call of *P. avia* is a brief, rapid, trill. The first call (Fig 6) consists in a series of seven consecutive pulses at a mean frequency of 561.5 Hz (541-572 Hz). The duration of the call is of 0.28 sec and duration between pulses of 0.020 sec. The initial and final pulses show the lesser intensity, lower than 80 dB, while the medium pulses have amplitude above 85 dB (Table 2, Fig. 6A).

The second call (Fig. 7) has nine consecutive pulses at a mean dominant frequency of 349.5 Hz (348-353 Hz). This call is longer than the first one (0.405 sec) and we are not sure if was emitted by the same individual. Each pulse has a mean duration of 0.032 sec and the duration between pulses is 0.011 sec. Like the first call, the first and last pulse were lower in intensity (under 60 dB), while the medium pulses presented amplitude above 62 dB (Table 2, Fig. 7B).

Discussion

The first exceptional thing about these observations is that this is the first case of underwater breeding behavior among hylids (with exception of Pseudinae, of which two genera are highly adapted to aquatic life).

A second noteworthy observation is that combat behavior has never been observed in *Plectrohyla*.

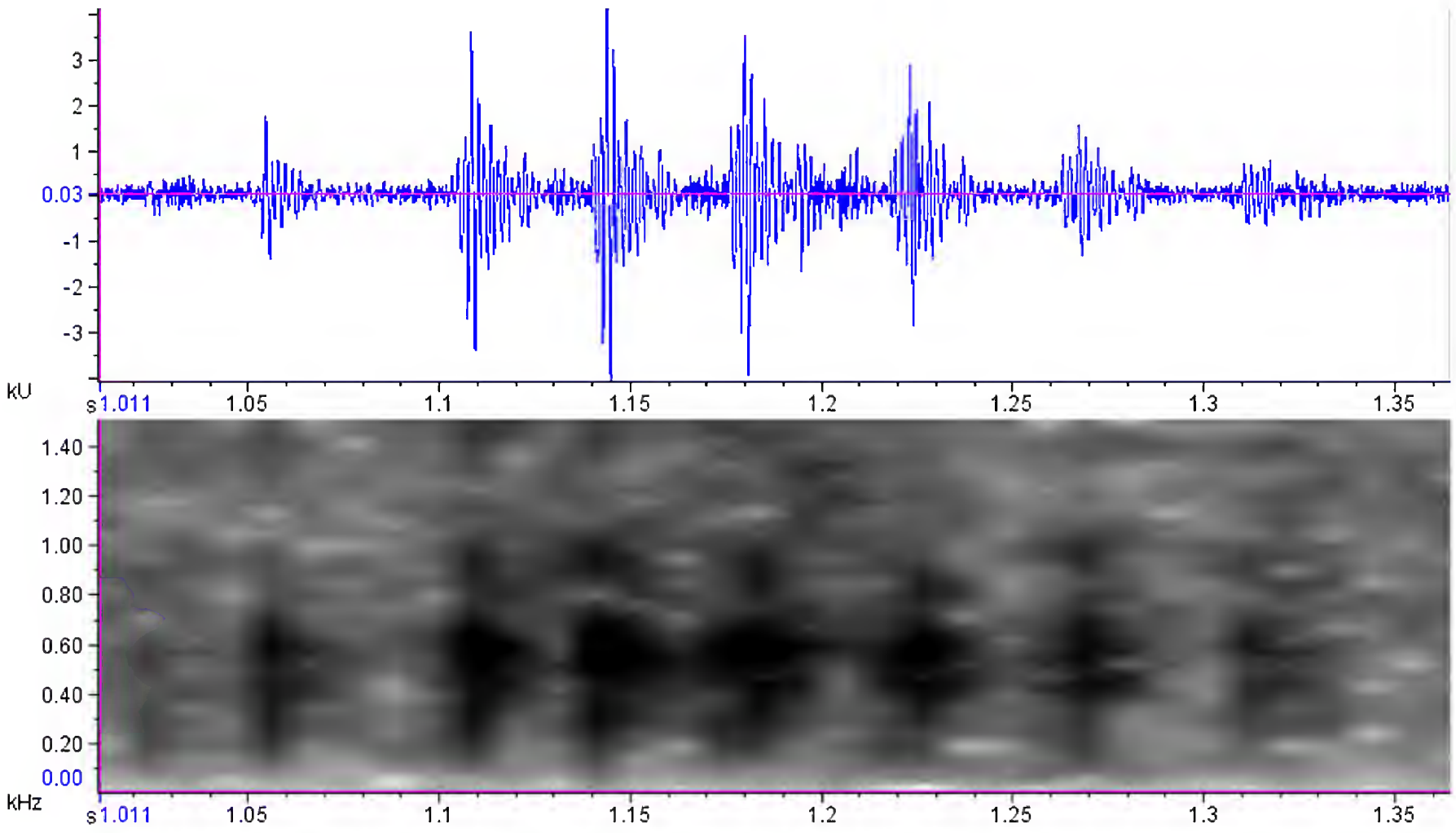

Fig. 6. Waveform (above) and spectrogram (below) of the first subaquatic call analyzed of *Plectrohyla avia*.

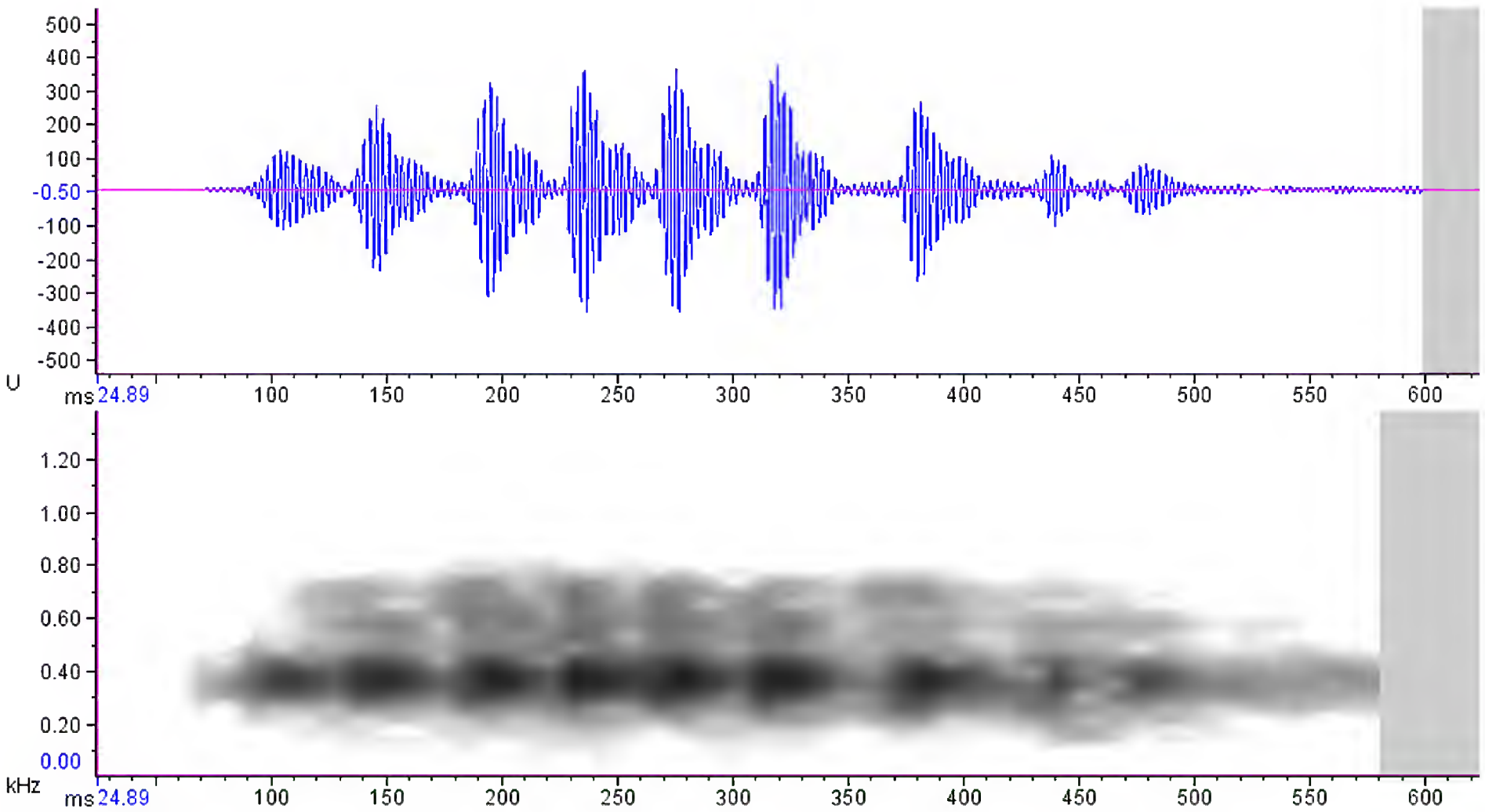

Fig. 7. Waveform (above) and spectrogram (below) of the second subaquatic call analyzed of *Plectrohyla avia*.

Dermal scratches, however, have been reported in some species (like *P. teuchestes* and *P. harwegi*; Duellman and Campbell 1992), and these observations suggest that they are caused by the prepollical spine used in some kind of combat. While large but stout teeth have been reported for *P. exquisita*, a very similar species (McCrannie and Wilson 1998), the teeth in the upper jaw in a breeding-condition male of *P. avia* (MZFC [Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autonoma de México, Mexico] 29273) are extraordinarily long, protruding from the upper jaw and curved (Fig. 8). This may have an important function to promote the female sexual reception or ovulation, as the male was seen apparently using them to scratch the female's head. However, we did not find any trace of mental gland on the reproductive male collected, and the function must not be similar to that in plethodontid salamanders, in which males also scratch female's head with its premaxillary teeth inoculating secretions from the mental glands (Arnold 1977; Duellman and Trueb 1994). Another possible explanation is that during the aquatic period, the individuals eat freshwater hard-shelled insects (Notonectidae, Hydrophilidae, and Gyrinidae). We did not observe any expansion of the throat on the calling male. Another striking feature was the presence of very well developed lateral skin soft folds (Fig 9), that may help in dermal respiration, as in high Andean frogs of the family Telmatobiidae (Duellman and Trueb 1994).

All reproductive activity that we witnessed was from 1715h to 1830h, when it was still light outside the dark ravine. We collected one male and came back after dark at 2200h, but not a single individual was observed in the area.

In the same ravine, many *Plectrohyla* cf. *sagorum* were heard and seen, including one amplectant pair. This species is smaller (males up to 45.5 mm, females up to 51.9 mm; Kohler 2010) and no individual was observed in the water; several males were calling from rocks facing the ravine, and an amplectant pair was on the wall of a small waterfall. In a nearby larger stream, with many *P. matudai* (including recent metamorphs) but not *P. avia*, we found four *Plectrohyla hartwegi*, close to a four m high waterfall, all were perched on stems and ferns in or close to, the spray zone. This species has very flared lips but no protruding teeth.

Fig. 8. Detail of the long and curved teeth protruding from the upper maxilla of a preserved reproductive male MZFC 29273. *Photo by Chris I. Grünwald.*

Fig. 9. Lateral view of a reproductive male MZFC 29273, showing the lateral skin folds. *Photo by César L. Barrio-Amorós.*

Conservation status

In less than two hours we saw eight *Plectrohyla avia* in a section of less than 20 m in the ravine. At the small pool and inside the holes in the wall of the canyon were many eggs attached to the rock or stems, some freshly laid, some with white embryos visible, and already some tadpoles recently hatched (Fig 10). We cannot be absolutely sure that all those eggs belong to *P. avia*, as we never saw females laying. This species, however, was the dominant in that sector of the ravine and occupied the lower pool (five individuals using the pool for reproductive purposes), and though no adults were seen in the upper pool (Fig. 10), two adult males were very close. On the upper pool only unpigmented eggs were seen, very likely belonging to *P. avia*.

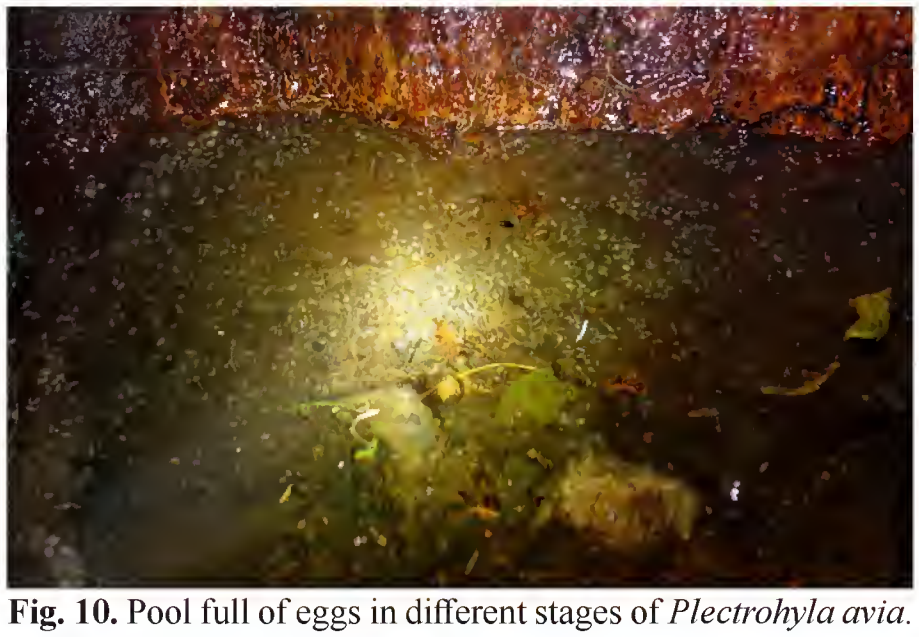

Fig. 10. Pool full of eggs in different stages of *Plectrohyla avia*. *Photo by César L. Barrio-Amorós.*

We queried local inhabitants about the frogs, and all them recognized the species, and said they are common. One boy told us that he enjoyed killing them. While the IUCN (Santos-Barrera et al. 2006) and Stuart et al. (2008) consider this species to be in the maximum category of vulnerability (CR A3e), Johnson et al (2015) consider the EVS score as 13 –of 20- (medium category). Probably the species is more widely distributed and more abundant than expected, but with a short period of activity at the beginning of the rainy season. More information is needed to establish a definitive category of conservation.

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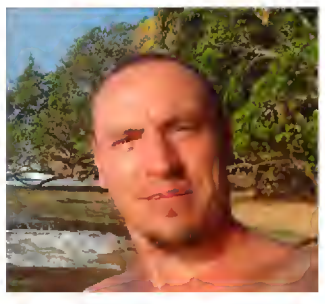
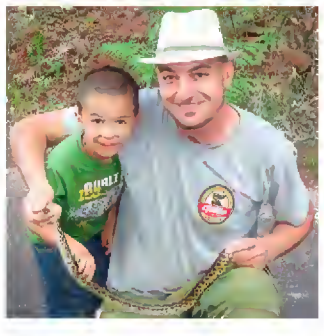


César L. Barrio Amorós is an anthropologist who has worked with herpetofauna in Spain, Venezuela, Peru, Costa Rica, and Mexico. His research interests include the biogeography and systematics of Amphibia and Reptilia, with emphasis in Dendrobatoidea and Terrarana from Venezuela, especially of the Guiana Shield. Now a resident of Costa Rica, he is a free-lance investigator and photographer. César has authored or co-authored more than 200 papers, including the description of 50 new species of amphibians and reptiles.



Christoph I. Grünwald is a German-Mexican herpetologist who for the last 15 years has led field expeditions to study herpetofauna. A specialist in Mexican biogeography, he has been involved with the discovery of over 100 range extensions and dozens of state records for amphibians and reptiles from around the country. Christoph specializes in rattlesnakes and pitvipers, and many important discoveries have involved this group. Currently he is leading research expeditions on Mexican rattleless pitvipers and direct-developing frogs. A co-founder of Biodiversa, A.C., an anti-extinction non-profit organization, Chris currently is developing a system of "micro-reserves," aimed at preserving the most vulnerable, high-endemism localities in Mexico.

Hector Franz-Chávez was born in Guadalajara, Mexico, and has had a passion for herpetology since childhood. He is a student of biology at the Universidad de Guadalajara (CUCBA), and his main interests include biogeography, natural history, and ecology of the herpetofauna of Mexico. He also is an avid nature photographer, and has collaborated in various herpetological inventories in different parts of Jalisco, and currently is working on an inclusive project on Mexican direct-developing frogs and several potentially new species of pitvipers. Hector has traveled extensively in the Sierra Madre Occidental and the Sierra Madre del Sur, where he has collected numerous specimens of interest.

Ángela M. Mendoza is a biologist from Universidad del Valle (Colombia) with a M.S. in biological sciences and is currently a Ph.D. student at the Universidad Nacional Autónoma de México (UNAM). Her work has focused mainly in the application of molecular tools in solving questions in ecology and conservation, with an emphasis in terrestrial vertebrates, mainly Neotropical amphibians.

Brandon La Forest was born in Phoenix, Arizona. He has been interested in herps, particularly vipers, as far back as he can remember. He studied Ecology and Evolutionary Biology at the University of Arizona in Tucson. A life long enthusiast, he enjoys traveling abroad to document reptiles and amphibians. Mexico has always been a special place for him; where he spends over a month each year collaborating with different biologist to catalog undocumented, undescribed, and under sampled reptiles and amphibians.